Position 181: Primer U (SEQ ID NO:73)

5'-TCTGTAATTTTTGGAGAAAGANNAGGATTGACAACGATAAATGTCAATGAAAAC-3'

N (22) = 50% C; 50% G

N (23) = 33.3% C; 33.3% G; 33.3% A

Position 182: Primer V (SEQ ID NO:74)

5'-TAATTTTTGGAGAAAGATGGNNATTGACAACGATAAATGTCAATGAAAAC-3'

N (22) = 50% C; 50% G

N (23) = 25% C; 25% G; 25% A; 25% T

Position 183: Primer W (SEQ ID NO:75)

5'-GTAATTTTTGGAGAAAGATGGGGANNAACAACGATAAATGTCAATGAAAAC-3'

N (25) = 50% C; 50% G

N (26) = 25% C; 25% G; 25% A; 25% T

Position 184: Primer X (SEQ ID NO:76)

5'-GTAATTTTTGGAGAAAGATGGGGATTGNNAACGATAAATGTCAATGAAAAC-3'

N (28) = 50% C; 50% G

N (29) = 25% C; 25% G; 25% A; 25% T

A PCR™ using the opposing primers H (SEQ ID NO:52) and F (SEQ ID NO:20) and plasmid pEG943 as a template was first performed to generate a DNA fragment containing the R148D and K219A mutations as well as the unique *Nhe*I restriction site marking the K219A mutation (FIG. 10). In order to generate *cry1C* fragments harboring loop α5-6 mutations, PCRs were run using a mutagenic primer (*e.g.*, primer R) and the opposing primer L (SEQ ID NO:64) (FIG. 11). The amplified DNA fragments were purified following agarose gel electrophoresis using the Geneclean II® procedure. For the overlap extension PCR™, approximately equimolar amounts of the two DNA fragments were mixed and amplified using the flanking primers H (SEQ ID NO:52) and L (SEQ ID NO:64). The amplification products were digested with the restriction enzymes *Bbu*I and *Age*I, the resulting *Bbu*I-*Age*I *cry1C* fragments subcloned into a *cry1C*

expression vector, and the *B. thuringiensis* EG10650 transformants constructed as described in Example 10. Table 16 summarizes the Cry1C mutants predicted from the mutagenesis procedure.

TABLE 16

SUMMARY OF CRY1C-R148D LOOP α5-6 MUTANTS

Amino Acid Position	Wild-Type Amino Acid	Primer	Predicted Amino Acid Substitutions	Mutant Designation
178	glycine	R	R, E, Q, A, G, P	Cry1C 1 -1, -2, -3, *etc.*
179	glutamic acid	S	R, E, Q, A, G, P	Cry1C 2 -1, -2, -3, *etc.*
180	arginine	T	R, E, Q, A, G, P	Cry1C 3 -1, -2, -3, *etc.*
181	tryptophan	U	R, E, Q, A, G, P	Cry1C 4 -1, -2, -3, *etc.*
182	glycine	V	R, E, Q, A, G, P, L, V	Cry1C 5 -1, -2, -3, *etc.*
183	leucine	W	R, E, Q, A, G, P, L, V	Cry1C 6 -1, -2, -3, *etc.*
184	threonine	X	R, E, Q, A, G, P, L, V	Cry1C 7 -1, -2, -3, *etc*

EXAMPLE 12 -- BIOASSAY EVALUATION OF CRY1C-R148D COMBINATORIAL MUTANTS

EG10650 transformants containing mutant *cry1C* genes were grown in C2 medium, the spore-crystal protein suspensions recovered, and one-dose bioassays performed against neonate larvae of *S. exigua* and *T. ni* as described in Example 4. Strain EG11832 (Cry1C-R148D) was used as the control strain in these bioassays. Dilutions of the spore-crystal suspensions were typically adjusted to obtain 20-40% mortality with strain EG11832. Replicated one-dose screens of the Cry1C-R148D combinatorial mutants identified several mutants with increased mortality. Sixteen of these mutants were grown again in C2 medium and their Cry1C crystal proteins quantified as described in Example 4. One-dose bioassays were performed against *S. exigua* using 50 ng Cry1C protein per diet well. One dose bioassays were performed against *T. ni* using 25 ng Cry1C protein per diet well. The results of those bioassays are shown in Table 17. Triplicate samples of the control strain EG11832 (Cry1C-R148D) were also tested. Several Cry1C-R148D combinatorial mutants show increased (approximately two-fold)

toxicity towards *S. exigua* when compared to EG11832 (Cry1C-R148D). Several of these mutants, including Cry1C 7-3, Cry1C 66-19, and Cry1C 69-24 also showed excellent toxicity towards *T. ni*.